# Global Transcriptomic Analysis of Chromium(VI) Exposure of *Desulfovibrio vulgaris* Hildenborough Under Sulfate-Reducing Conditions

K-052

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**Abstract**

*Desulfovibrio vulgaris* is an anaerobic sulfate-reducing bacterium (SRB) able to reduce toxic heavy metals such as chromium and uranium, and *D. vulgaris* represents a useful SRB model for the bioremediation of heavy metal contamination. Although much work has focused on Cr and U reduction via individual enzymes, less is known about the cellular response to heavy metal stress in *Desulfovibrio* species. Cells were cultivated in a defined medium with lactate and sulfate, and a sub-lethal concentration of Cr(VI) was added at mid-exponential phase growth. The growth was affected upon addition of Cr(VI), but the treated culture had a similar growth rate to no-treatment culture within approximately 6 h. The major differentially expressed genes included those coding for a presumptive FMN reductase, an hsp20-like protein, a facilitator, an ArsR-like regulator, and a predicted carboxynorspermidine decarboxylase (*nspC*). A presumptive permease gene was in a predicted operon with the ArsR-like gene, and the permease gene displayed an upward trend of expression during the first 2 h of Cr exposure. At 60 minute post-treatment, genes for a nitroreductase, thioredoxin reductase, and Clp protease adaptor were up-expressed. Presumptive genes for agmatinase, flocculin, Zn-chelator, and a *kdpC* were also up-expressed. In addition, four predicted metal or drug transporter genes were up-expressed, and included presumptive *mer*P, *acr*A, and *chr*A. Interestingly, six up-expressed genes were on the megaplasmid, and included hypothetical proteins, a presumptive facilitator, and a predicted *chr*A (chromate transporter). These results suggested a role of megaplasmid-encoded proteins that may be important to reduce chromate accumulation in the cell. A strain that was missing the megaplasmid was more susceptible to chromium exposure, and this result corroborated the microarray data. Further work is needed to delineate the possible roles of the respective genes.

**Introduction**

A number of anthropogenic activities have caused extensive Cr contamination in both soils and water. Cr is the third most common pollutant at hazardous waste sites and the second most common inorganic contaminant after Pb. Cr(VI) is water-soluble, mutagenic, and carcinogenic, but Cr(III) is less-soluble, less-toxic, and less mobile. A variety of studies have documented the ability of SRB, including *Desulfovibrio* spp., to reduce toxic metals such as U(VI) and Cr(VI) enzymatically, a process that results in the production of less water-soluble species. The modification of solubility properties caused by changing the redox state of the metal presents itself as a potential avenue for bioremediation of contaminated groundwater and soils (Lloyd, 2003). Previous research specifically points toward SRB as environmentally-relevant experimental systems for the study of heavy metal and radionuclide reduction. Sulfate-reducers provide several advantages with respect to heavy-metal reduction including the presence of sulfate in a variety of environments and the protection of immobilized heavy metals from oxidation with iron sulfides (Abdelouas et al., 2000).

Previous work has shown that *D. vulgaris* requires hydrogen sulfide, hydrogenases and cytochrome *c3* for the reduction of Cr(VI) (Chardin et al., 2002), and that Cr(III) can be detected on the cell surface as well as between the cytoplasmic and outer membranes (Goulhen et al., 2005). Microcalorimetry was used to observe energy production without growth in the presence of Cr(VI) (Chardin et al., 2002), but acetate and sulfate levels were not reported. In addition, the re-establishment of growth was not monitored, and the growth medium was not defined. In comparison, recent work has shown that U(VI) inhibited sulfate-depletion, and that both Fe(III) and U(VI) inhibited lactate-mediated sulfate reduction (Elias et al., 2004). However, it is not known if a lag time ensues with respect to cell growth post-treatment with U(VI) or Fe(III).

Abdelouas et al. 2000. Biological reduction of uranium in groundwater and subsurface soil. Sci. Total Environ. 250:21-35.

Brown et al. 2006. Molecular Dynamics of the *Shewanella oneidensis* Response to Chromate Stress. Molecul. Cellul. Proteomics (DOI 10.1074/mcp.M500394-MCP200).

Chardin et al. 2002. Bioremediation of chromate: thermodynamic analysis of the effects of Cr(VI) on sulfate-reducing bacteria. Appl. Microbiol. Biotechnol. 60:352–360.

Elias et al. 2004. Periplasmic cytochrome *c3* of *Desulfovibrio vulgaris* is directly involved in H2-mediated metal but not sulfate reduction. Appl. Environ. Microbiol. 70:413–420.

Goulhen et al. 2005. Cr(VI) detoxification by *Desulfovibrio vulgaris* strain Hildenborough: microbe–metal interactions studies. Appl. Microbiol. Biotechnol. (DOI 10.1007/s00253-005-0211-7).

Lloyd, J.R. 2003. Microbial reduction of metals and radionuclides. FEMS Microbiol. Rev. 27:411-425.

### Growth Effects of Different Cr(VI) Levels

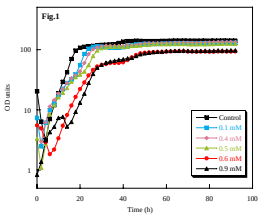



**Figure 1**. Cells were grown in various levels of Cr(VI) from 0.1 mM to 0.9 mM. At concentrations between 0.1 mM to 0.5 mM Cr(VI), initial growth was not affected, slowed transiently, and then reached a similar final OD as untreated cells. At 0.6 mM Cr(VI), cells lagged transiently, and then reached a similar growth rate as untreated cells. However, final OD was lower. Between 0.7 and 0.9 mM Cr(VI), the lag time increased slightly. The concentration of 0.55 mM Cr(VI) was used for transcriptomic analyses.

### Protein Levels After Cr-Treatment

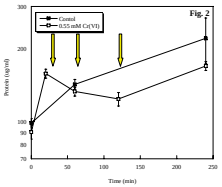



**Figure 2**. Cells were incubated with sulfate and lactate until an optical density (600 nm) of approximately 0.3 was reached. Cr(VI) was added to a final concentration of 0.55 mM, and biomass was harvested over time (denoted by arrows) for transcriptomic and FTIR analysis. The protein levels indicated that cell growth initially increased, lagged, and then reach a growth rate similar to untreated cells.

### FTIR After Cr-Treatment

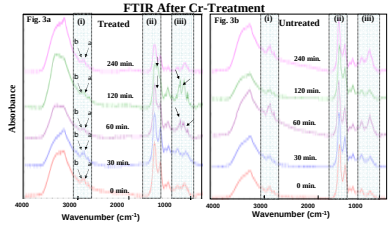



**Figure 3a and b**. Significant structural and functional changes were observed in biological molecules of live *D. vulgaris* cells within an hour of Cr(VI) treatment (**Fig. 3a**). (**i**) A change in the membrane lipid composition/structure as indicated by the decrease in the ratio of the infrared absorption intensity centered at ~2925 $cm^{-1}$ ($CH_2$ asymmetric stretching vibrations; **a**) to that at ~2958 $cm^{-1}$ ($CH_3$ asymmetric stretching vibrations; **b**). This suggested a decrease in acyl chain length and/or a decrease in fatty acid content. (**ii**) A change in secondary protein structures was indicated by the changes in the spectral character centered at the amide I of α-helical and β-sheet protein structures (arrows). (**iii**) Drastic structural and functional changes were detected for the $PO_2^-$ groups in nucleic acids and the C-O-C and C-O-P groups in various oligo- and polysaccharides (arrows). Most of the prominent spectral features induced by the Cr(VI) treatment decreased 4 h post-treatment. (Note: all spectra were shifted vertically for clear viewing.)

### Number of Genes with Altered Expression

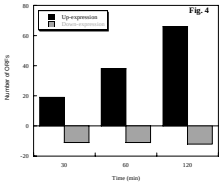



**Figure 4**. During the 2 h post-treatment, the number of up-expressed genes increased. However, during the same time period, the number of down-expressed genes remained largely unchanged. Almost half of the up-expressed genes at 120 min post-treatment were hypothetical or conserved hypothetical proteins.

### Up-Expression of Megaplasmid ORFs in Response to Cr(VI)

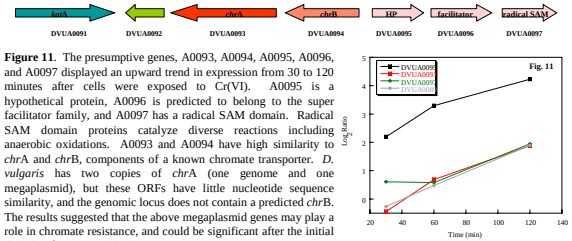



**Figure 11**. The presumptive genes, A0093, A0094, A0095, A0096, and A0097 displayed an upward trend in expression from 30 to 120 minutes after cells were exposed to Cr(VI). A0095 is a hypothetical protein, A0096 is predicted to belong to the super facilitator family, and A0097 has a radical SAM domain. Radical SAM domain proteins catalyze diverse reactions including anaerobic oxidations. A0093 and A0094 have high similarity to *chr*A and *chr*B, components of a known chromate transporter. *D. vulgaris* has two copies of *chr*A (one genome and one megaplasmid), but these ORFs have little nucleotide sequence similarity, and the genomic locus does not contain a predicted *chr*B. The results suggested that the above megaplasmid genes may play a role in chromate resistance, and could be significant after the initial Cr(VI) reduction to Cr(III).

### Phage Genes

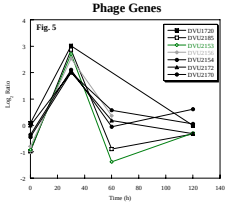



**Figure 5**. Almost half of the significantly up-expressed genes 30 min post-treatment were predicted to be phage genes. The genes then displayed down-expression at the later time points. The results suggested that an initial response was elicited by the Cr-stress b
y part of the phageome; however, the genes were subsequently down-expressed as the cells recovered.

### Reductase/Dehydrogenase

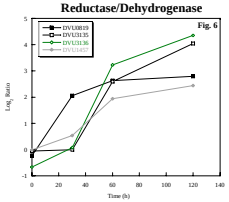



**Figure 6**. The following genes displayed up-expression at all time points post-treatment: FMN reductase (DVU0819); NADPH dehydrogenase (DVU3135); nitroreductase (DVU3136); thioredoxin reductase (DVU1457). A nitroreductase has been reported to be up-expressed in *Shewanella oneidensis* in response to Cr(VI) (Brown et al., 2006).

### Heavy Metal Resistance

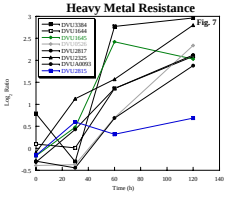



**Figure 7**. Genes predicted to be involved in heavy-metal resistance for *D. vulgaris* displayed up-expression for approximately 1 h post-treatment with 0.55 mM Cr(VI). The expression of the following genes are dentoed above: Zn-resistance protein (DVU3384); putative permease (DVU1644); transcriptional regulator (arsA; DVU1645); drug resistance transporter (DVU0526); multidrug resistance protein (DVU2817); OM efflux protein (DVU2815); mercuric transport component (DVU2325); chromate transport (DVUA0093).

### Response-Regulator and Protease

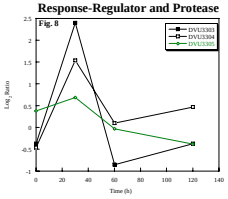



**Figure 8**. DVU3303 is an annotated Lon protease that also contains a putative AAA-ATPase domain. The Lon proteases are serine proteases involved in protein turnover-typically during heat-shock. DVU3303 is also up-expressed during heat-shock in *D. vulgaris*. DVU3304 and DVU3305 are predicted to be in an operon with DVU3303 are a putative histidine kinase and response regulator, respectively.

### Cellular Response to Cr(VI) Differs Between Wild-Type and ΔMP

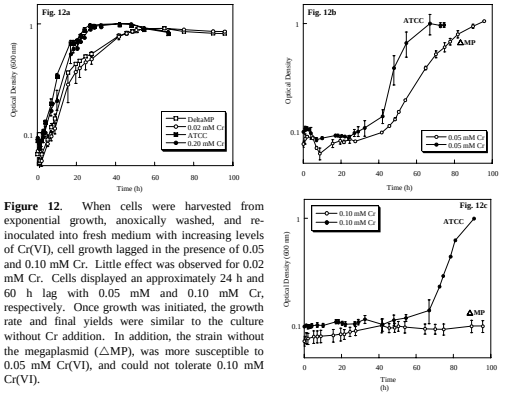
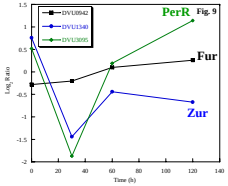



**Figure 12**. When cells were harvested from exponential growth, anoxically washed, and re-inoculated into fresh medium with increasing levels of Cr(VI), cell growth lagged in the presence of 0.05 and 0.10 mM Cr. Little effect was observed for 0.02 mM Cr. Cells displayed an approximately 24 h and 60 h lag with 0.05 mM and 0.10 mM Cr, respectively. Once growth was initiated, the growth rate and final yields were similar to the culture without Cr addition. In addition, the strain without the megaplasmid (ΔMP), was more susceptible to 0.05 mM Cr(VI), and could not tolerate 0.10 mM Cr(VI).

### PerR, Fur, and Zur




### Predicted PerR Regulon

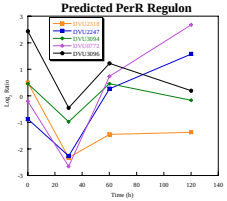



**Figure 9**. The putative regulators PerR, Fur, and Zur responded differently to the Cr(VI) stress. Expression levels for Fur did not change significantly, but expression of PerR and Zur decreased at the 30 min post-treatment. The levels of PerR and Zur then increased at subsequent time points. The following genes predicted to be part of the PerR regulon displayed similar trends in expression as PerR: rubrerythrin (DVU2318); alkyl hydroperoxide reductase C (DVU2247); rubrerythrin (DVU3094); hypothetical protein (DVU0772); and rubrerythrin (DVU3094).

### Sulfate Permeases

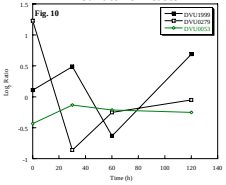



**Figure 10**. Chromate has been shown to enter bacterial cells via sulfate permeases. *D. vulgaris* has three predicted sulfate permeases (DVU1999, DVU0279, and DVU0053). The putative sulfate permease genes did not show significant changes in expression levels; however, DVU0279 did show a trend in decreased expression upon Cr(VI) treatment.

### Hypothetical Model Based Upon Up-Expression of Annotated Genes for Cr(VI) Response

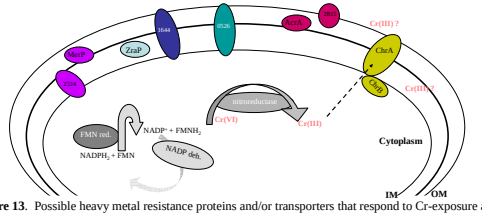



**Figure 13**. Possible heavy metal resistance proteins and/or transporters that respond to Cr-exposure are shown in Figure 13. Location of putative proteins based upon amino acid sequence predictions. Genes for the displayed proteins were up-expressed during Cr(VI) and/or Cr(III) exposure. Putative proteins denoted by the same color are predicted to interact and are located in operons. The FMN-dependent nitroreductase might reduce Cr(VI) directly or reduce a Cr-complex. The FMN reductase could synthesize $FMNH_2$ and the NADP dehydrogenase might be used to regenerate $NADPH_2$. The *chr*AB genes on the megaplasmid most likely play a key role in Cr(III) efflux based upon microarray data and growth data. Additional toxicological effects could be occurring once the Cr(III) is produced via protein denaturation in the cytoplasm, periplasm, and outer cell proper.

DVU3384 – Zn-resistance
DVU0526 – drug resistance
DVU2325 – Hg-resistance
DVU2324 – metal ATPase
DVU1644 – putative permease
DVU2817 – multidrug resistance
DVU2815 – OM efflux
DVU0819 – FMN reductase
DVU3135 – NADP dehydrogenase
DVU3136 – nitroreductase (FMN)
DVUA0093 - chrA
DVUA0094 - chrB

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